

## NOTES

### Protein Domains and Residues Involved in the CheZ/CheA<sub>S</sub> Interaction<sup>▽</sup>

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**CheZ localizes to chemoreceptor patches by binding CheA-short (CheA<sub>S</sub>). Residues 70 to 134 of CheZ, constituting the apical loops and part of the dimerization domain, suffice for localization. Replacements of Tyr-118, Ile-119, Leu-123, Arg-124, and Leu-126 of CheA interfere with localization. These residues are exposed in the 'P1 domain of CheA<sub>S</sub>.**

CheZ is the phosphorylated CheY (CheY-P) phosphatase of *Escherichia coli* (5) and a number of other gram-negative bacteria (2, 3). *E. coli* CheZ forms a homodimer that binds two molecules of CheY-P (Fig. 1A) (20). Within each subunit, there is an N-terminal helix of about 30 residues that is involved in negative control of phosphatase activity (16). As CheZ is depicted in Fig. 1, following this helix are a sharp turn, an extended ascending helix, a hairpin loop, and an extended descending helix. The Gln-147 residues at the active sites are in the middle of the extended helical region. Following the descending helix, there is an unstructured flexible connector to the short C-terminal helix that constitutes a CheY-P binding site.

Shortly above the active sites, the two hairpin loops splay out from each other. The Trp-97 and Phe-98 residues that are important for localization but not for dimerization or activity (3, 19) are located near the apical loops of CheZ. Ser substitutions for hydrophobic residues Trp-94 through Val-121 block localization but also interfere with chemotaxis (3), perhaps because they interfere with dimer formation.

A short form of the CheA kinase (CheA<sub>S</sub>) (7) begins with Met-98 of the long form of CheA (CheA<sub>L</sub>). CheA<sub>S</sub> is produced in a 1:2 ratio relative to CheA<sub>L</sub> (17) and is required for the localization of CheZ to the receptor patch. CheA<sub>S</sub> is not essential for normal chemotaxis, as assessed in standard laboratory assays (15). However, both theoretical calculations (9, 10) and in vivo measurements (19) indicate that cells that fail to localize CheZ have very different intracellular distributions of CheY-P than wild-type cells. CheZ-localizing strains show a very abrupt decline in the CheY-P concentration near the receptor patch and rather uniform levels throughout the rest of the cell. Cells in which CheZ does not localize show a gradual decline in the CheY-P concentration with distance from the receptor patch, so that CheY-P concentrations vary throughout the cell.

**The apical hairpin region of CheZ plus a flanking portion of the dimerization domain suffice for polar localization.** To determine the minimal portion of CheZ required for polar localization, CheZ proteins with increasingly large deletions at the amino-terminal and carboxyl-terminal ends were fused to green fluorescent protein (GFP) (Fig. 2). Each plasmid-encoded fusion protein was expressed from plasmid pBJC104 by induction with IPTG (isopropyl-β-D-thiogalactopyranoside) at 10 μM, the concentration that gave the best chemotaxis when full-length CheZ-GFP was expressed from the same plasmid in a Δ*cheZ* strain (data not shown). Patterns of localization were observed by fluorescence microscopy (3).

A region of 64 residues (70 to 133) that includes the apical hairpin turn (residues 100 to 104) and flanking sequences was sufficient for localization to the cell pole. A smaller fragment, comprising residues 81 to 121, did not localize GFP. Residues 70 to 83 and 121 to 134 are at the top of the four-helix bundle that forms the core of the CheZ structure, and they form critical contacts between the two subunits (Fig. 1A). These data suggest that all of the determinants required for polar localization are restricted to the area previously identified by mutagenesis (3) plus enough flanking sequence to allow dimerization.

**Hydrophobic residues in the CheA<sub>S</sub> 'P1 domain are required for localization of CheZ-GFP.** The crystal structure (12) of the isolated CheA P1 phosphotransfer domain (21) from *Salmonella enterica* reveals a four-helix bundle plus an additional amphipathic helix that lies adjacent and antiparallel to the fourth helix (Fig. 1B) (13). Met-98 is located near the carboxyl-terminal end of helix 4, leaving the hydrophobic face of helix 5 in CheA<sub>S</sub> exposed to solvent. This fragment of the P1 domain has been named 'P1.

The aliphatic and aromatic residues of helix 5 were converted to Ala and Ser by site-directed mutagenesis in *cheA* carried on plasmid pBJC200, made by cloning the PCR-amplified *cheA* gene into plasmid pLC112 (1). The Arg-124 residue was also converted to Glu. Mutations were confirmed by DNA sequencing. The plasmids encoding mutant proteins were introduced into Δ*cheA* strain BC206, which expresses wild-type CheZ-GFP from the *λatt* chromosomal locus (3). Cells were grown to late exponential phase at 30°C in tryptone broth (11)

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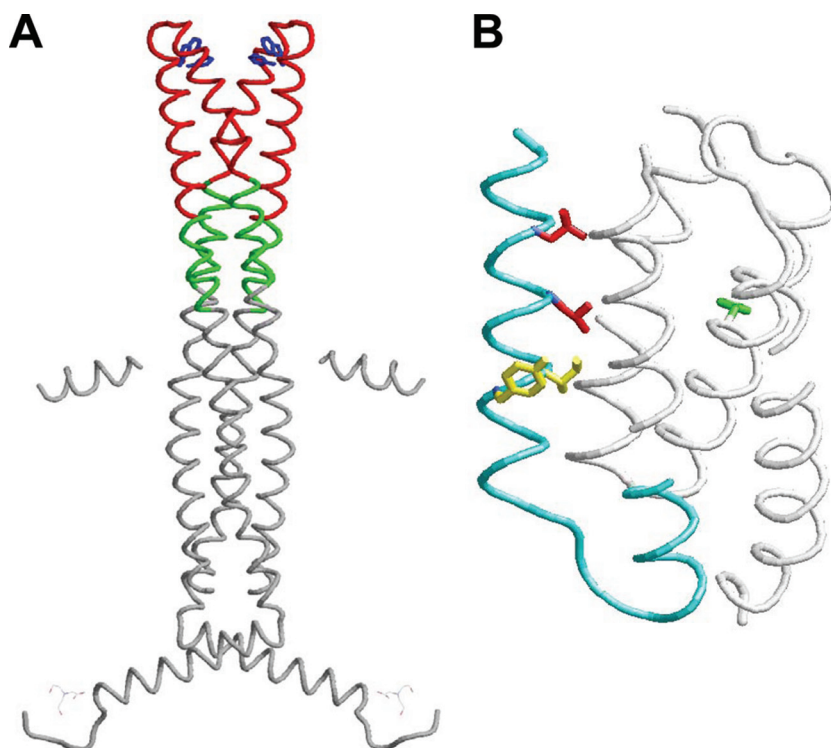


FIG. 1. Regions of CheZ and CheA<sub>S</sub> required for localization of CheZ to receptor patches. (A) A backbone trace of CheZ from the CheZ-CheY cocrystal (20) is shown. Residues Try-97 and Phe-98, mutations of which specifically inhibit CheZ-GFP localization, are shown in blue. The portion of CheZ shown in red is not sufficient to localize CheZ-GFP fusions to receptor patches. The addition of the portion of CheZ shown in green enables the CheZ-GFP fusion protein to localize to receptor patches. The disconnected helices represent the peptides that bind CheY-P and present it to the active site. (B) Backbone trace of the *Salmonella* CheA P1 crystal structure (12). The images shown were created from the coordinates provided in the Protein Data Bank entry for identification number 1I5N by using RasTop. The part of the P1 domain retained in 'P1 is shown in cyan. The residue at the site of phosphorylation, His-48, is shown in green. Ala or Ser substitutions for Leu-123 and Leu-126 (shown in red) strongly inhibit CheZ-GFP localization, whereas an Ala or Ser substitution for Ile-119 (shown in yellow) and the Ser substitution for Phe-118 (also yellow) moderately affect localization.

with 100  $\mu$ M sodium salicylate and 1 mM IPTG added to induce the expression of CheA and CheZ-GFP, respectively. These conditions allow good chemotaxis in tryptone semisolid (0.325%) agar and produce levels of CheA that are comparable to those produced from the chromosomal *cheA* gene expressed from the native *mocha* operon promoter. Fluorescent images of cells expressing different CheA proteins were captured digitally (3), and the effects of each mutation on CheZ localization were quantified by determining the percentage of cells with receptor patches.

In a strain expressing wild-type CheA, 75 to 80% of the cells had at least one visible receptor patch (Fig. 3). The L123A, L123S, and L126A substitutions (L126S was not obtained) dramatically reduced CheZ-GFP localization;  $\leq 20\%$  of the cells had visible patches. Proteins with the Y118S, I119A, and I119S substitutions exhibited intermediate localization defects, whereas CheZ-GFP with the F118A substitution still localized normally. The R124E substitution also produced moderate localization defects, but a Ser substitution for Phe-116 or Leu-128 had no significant effects on CheZ-GFP localization. Chemotaxis, as assessed by swarm ring formation in tryptone semisolid agar, was normal for each strain expressing a mutant protein. All of the plasmid-encoded CheA proteins were present in the same amount as plasma-encoded wild-type

CheA, as determined by immunoblot analysis using anti-CheA antibody (data not shown). The ratios of full-length CheA to CheA<sub>S</sub> were also normal for all of the mutants.

**Conclusions.** This study defines the minimum portion of CheZ required for polar localization and indicates that certain residues on the hydrophobic face of amphipathic helix 5 of the P1 domain are crucial for interaction with CheZ. These conclusions are in keeping with the conservation of the CheZ apical region and the 'P1 domain in the enteric bacteria (3). In contrast, these two regions are highly variable in strains in which CheZ does not localize to receptor patches (3).

The nuclear magnetic resonance structure presented in the accompanying report by Hao et al. (4) shows the apical loops of the CheZ dimer interacting with the hydrophobic face of helix 5 of the 'P1 domain. This structure suggests that the oligomerization of CheA<sub>S</sub> through the 'P1 domain that was seen in an equilibrium sedimentation analysis (8) is not required for the interaction of CheZ and CheA<sub>S</sub> and may even prevent it. In the nuclear magnetic resonance structure, the residues in CheZ (3) and CheA<sub>S</sub> (14; this study) that are crucial for the interaction of the two proteins are in close proximity.

The findings of a recent study (6) using fluorescence energy resonance transfer to measure interactions of chemotaxis pro-

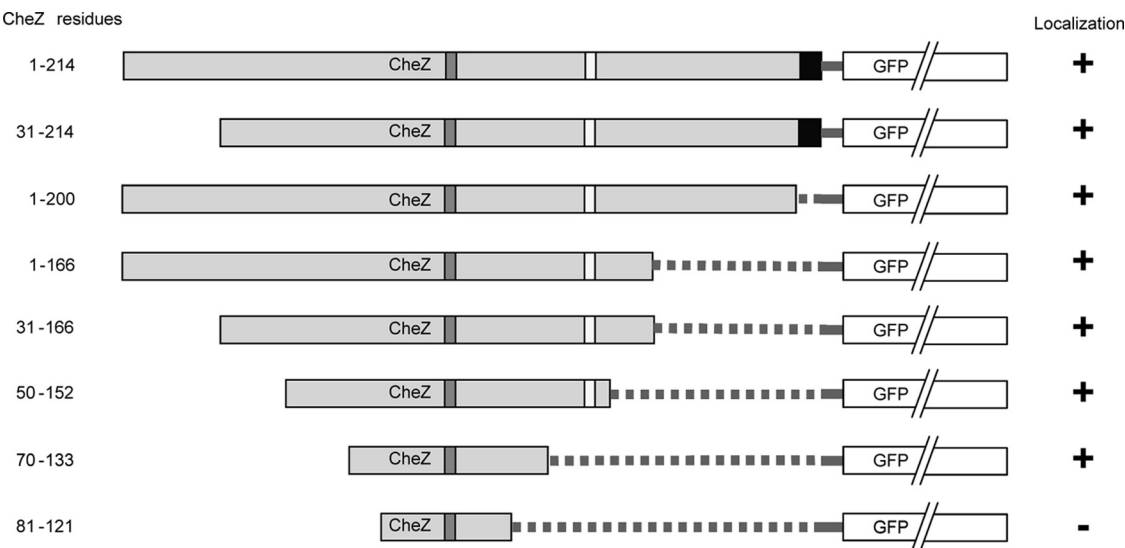


FIG. 2. The apical loop region of CheZ contains all determinants required for the localization of CheZ to the receptor patch. Fragments of *cheZ* corresponding to products with N-terminal and C-terminal deletions were fused to *gfp* by replacing the *cheZ* sequences in the *cheZ-gfp* gene fusion on plasmid pBJC104 (3). The CheZ residues encoded by each gene fusion are shown and represented diagrammatically. + indicates a pattern of localization indistinguishable from that of full-length CheZ-GFP (3), in which 70 to 90% of the cells contain fluorescent patches of CheZ-GFP. - indicates that no localized patches of CheZ-GFP fluorescence were observed. There were no intermediate patterns of localization among the constructs examined in this study. The dark gray bar indicates the positions of Trp-97 and Phe-98. The thin white bar shows the position of the key active-site residue, Gln-147 (20), and the black box shows the location of the C-terminal peptide that binds CheY-P.

teins in vivo indicate that CheZ can also interact with the P1 domain of CheA<sub>L</sub>, albeit with lower affinity than with CheA<sub>S</sub>. It is unclear whether this association is biologically significant, and it is also unclear whether the intact P1 domain must unfold

to some degree to expose helix 5 for CheZ to bind. This result does suggest, however, that at least some CheZ may localize to the receptor patch in the absence of CheA<sub>S</sub>.

The difference in distribution of CheY-P in CheZ-localizing

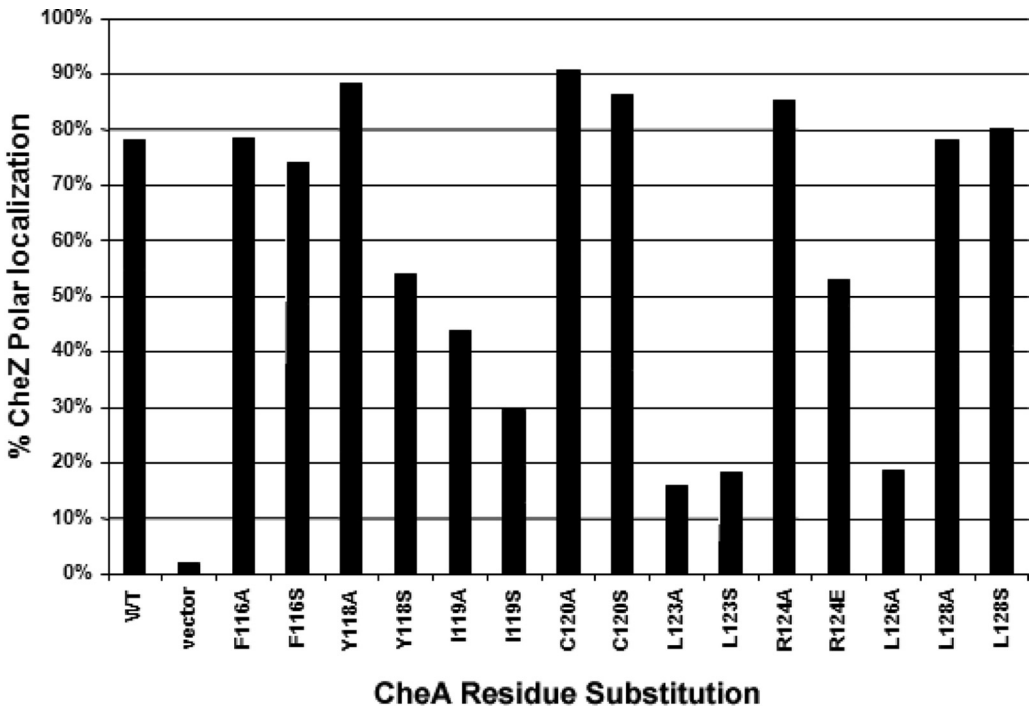


FIG. 3. Formation of receptor patches in strains expressing different substitutions in the 'P1 domain of CheA<sub>S</sub>. RP9353 ( $\Delta cheA$ ) cells expressing various variants of CheA and CheZ-GFP were examined by fluorescence microscopy. The ability of each CheA species to mediate the localization of CheZ-GFP to receptor patches was quantified as the percentage of cells showing one or more receptor patches.

and -nonlocalizing cells (9, 10, 17) suggests a function for polar localization. In enteric bacteria, peritrichous flagella arise at essentially random points on the cell surface at different distances from the receptor patch. The ability to maintain CheY-P at nearly constant levels throughout the cell exposes all flagella to similar concentrations of CheY-P, and all flagella should have similar clockwise/counterclockwise rotational biases. Changes in CheY-P production at the receptor patch may also propagate more rapidly and evenly through the cell when CheZ is concentrated at the receptor patch, thereby improving performance in chemotaxis.

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